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The effect of 5% NaOCl and 17% EDTA, at 24hrs and 8days, on the microhardness of MTA, Biodentine and Pozzolan cement: An in-vitro study.

ABSTRACT:

Aim & Objectives: The aim of this study was to evaluate the effects, at 24 h and 8 days, of 5% NaOCl and 17% EDTA on the Vicker's microhardness of Mineral Trioxide Aggregate(MTA Angelus) (MTAA), Biodentine(Septodont, Saint Maur des Fosse's France) and Pozzolan based endodontic cement named Endocem MTA(Maruchi, Wonju, Korea).

Materials and method: Sixty samples of MTAA, Biodentine and Endocem MTA were tested for baseline microhardness at 24 h. They were divided into 12 subgroups (5% NaOCl or 17% EDTA, 24 h and 5% NaOCl or 17% EDTA at 8 days) and microhardness was evaluated at different time points. Results were recorded and analysed statistically via one-way ANOVA and Tukey's post hoc test.

Results: MTAA had a higher baseline microhardness than both biodentine and Endocem MTA. At 24 hrs, the microhardness of all the materials was reduced by NaOCl and EDTA. At 8 days, NaOCl reduced the microhardness of MTA but that of Biodentine and Endocem MTA was increased. EDTA at 8 days, reduced the microhardness of both MTAA and Biodentine but an increase was seen with Endocem MTA.

Conclusion: Changes in microhardness of MTAA, Biodentine and Pozzolan cement(Endocem MTA) were associated with the time for which the materials are allowed to set as well as the irrigating agent used.

Keywords: Perforation repair materials, irrigants, chelating agents, vicker's microhardness.

Introduction:

Perforation during endodontic procedure has been reported to be a major factor in up to 9.6% of endodontic failures.[1] These are reported to be the second most common cause of failure in endodontics. Ideally, perforations should be repaired with an appropriate material as soon as possible, preferably in the same appointment for a better prognosis.[2] An ideal perforation repair material should be:

(a) biocompatible
(b) non-toxic
(c) non-cariogenic
(d) easy to place
(e) bacteriostatic
(f) radiopaque
(g) relatively inexpensive
(h) should provide adequate seal
(i) have ability to produce osteogenesis and cementogenesis
(j) beneficial to be used as a resorbable matrix in which a sealing material can be condensed.

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The materials that can be used for root perforation repair are: MTA(e.g. MTA Angelus White (Angelus, Londrina, Brazil) (MTAA), Biodentine and Pozzolan cement (Endocem MTA).[3]

MTA (Mineral Trioxide Aggregate):

Mineral Trioxide Aggregate is regarded as the gold standard material for perforation repair because of its superior sealing

[1]DASANI, S., [2]HORA, B. S., [3]AJMERA , R. S., [4]GUPTA, B., [5]DONDULKAR, Y., [6]KOSTA , S.
[1,2,3,5]Dept. of Conservative Dentistry & Endodontics, Chhattisgarh Dental College and Research Institute, Rajnandgaon, Chhattisgarh
[4]Brijesh Gupta (Reader, Dept. of Oral & Maxillofacial Surgery, Institute of Dental Education & Advance Studies, Gwalior, Madhya Pradesh)
[6]Academician & Private Practitioneer

Address for Corresponding : Dr. Baljeet Singh Hora
Department of Conservative Dentistry & Endodontics
Chhattisgarh Dental College and Research Institute,
Sundra, Rajnandgaon, Chhattisgarh, 491441
E-mail: drbaljeet69@yahoo.com

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ability and biocompatibility.[2] It is composed of Calcium oxide (CaO), Silicon dioxide (SiO_2), Bismuth trioxide (Bi_2O_3), Aluminium oxide (Al_2O_3), Magnesium oxide (MgO), Sulfur trioxide (SO_3), Chlorine (Cl), Ferrous oxide (FeO), Phosphorus pentoxide (P_2O_5), Titanium dioxide (TiO_2), Carbonic acid. But its long setting time poses great challenge to single visit endodontic treatment for such cases. The mean setting time for MTA is 165±5min.[4]. MTA retention and push-out strength increase with time, extending from 72 hours to 21 days, indicating a prolonged maturation process of the material.5 Washout of unset MTA can occur if the unset material is subjected to immediate irrigation.[6,7] The recall period after repairing the perforation is also not standardized by various clinicians. It ranges from 24 hrs to almost one week.[8, 9, 10, 11]

Biodentine:

Biodentine(BD), a new bioactive calcium silicate-based cement has been recently launched in the dental market as a 'dentin substitute'. It is available as powder and liquid as an accelerator. It is mainly composed of Tricalcium silicate ($3CaO.SiO_2$) (main core material), Dicalcium silicate ($2CaO.SiO_2$) (second core material), Calcium carbonate ($CaCO_3$)(filler), Zirconium Oxide (ZrO_2) (radio-opacifier) and Iron oxide(colouring agent). The presence of setting accelerator $CaCl^{o}$ in the liquid of Biodentine results in faster setting thereby improving its handling properties and strength. This is an advantage over MTA. Its setting time is 10 minutes. Also the flexural strength of set biodentine is much higher as compared to MTA.[12] Ballal et al. evaluated the effect of various acids on the microhardness, surface topography and push out bond strength of BD to root canal dentin. They reported that maleic acid, EDTA and phosphoric acid reduced the microhardness of Biodentine.[13] Guneser et al. have evaluated the influence of different root canal irrigating solutions on the push out bond strength of BD and MTA. They concluded that BD demonstrated a considerable performance after being exposed to root canal irrigants as compared to MTA.[14]

Pozzolan Cement (Endocem Mta):

A pozzolan-based bioactive endodontic cement named Endocem MTA (Maruchi, Wonju, Korea) was recently introduced to the dental market. The manufacturer claims that Endocem MTA has rapid setting time up to 4 minutes and easier manipulation properties with a chemical composition comparable to that of commercially available portland-based MTA. Unhydrated Endocem MTA is composed primarily of tricalcium silicate, dicalcium silicate and bismuth oxide.[15] Gan-Yeon Jang et al. investigated the effects of various root canal irrigants on "washout" of the MTA-derived pozzolan cement & ProRoot MTA in a furcal perforation model. The results of the study were ProRoot showed higher washout scores than Endocem under all irrigation solutions.[16] There is scarce evidence regarding the recommendation of ideal time to leave the pozzolan material to set before setting it to irrigation during root canal treatment.

Also there is limited research work in the literature comparing the effects of irrigants on completely set MTA , Biodentine and pozzolan cement. So such study done to compare the effects of irrigants on set MTA, Biodentine and Pozzolan cement could be contributory in determining an appropriate recall period for the continuation of root canal treatment after the placement of the perforation repair material.

So, the aim of this study was to evaluate and compare, at 24hrs and 8days, the effects of 5% NaOCl and 17% EDTA as irrigants on the microhardness of MTA, Biodentine and Pozzolan cement.

Materials And Method:

The materials used in this study are shown in Fig 1.:

Fig 1: Test materials and irrigants used

A 3-mm diameter cylindrical diamond bur was used to drill six identical wells in ten 5-mm thick acrylic sheets(Fig 2). The sheets were placed on and sealed against another sheet to prevent material pushout. MTAA, Biodentine and Pozzolan cement were mixed in accordance with the manufacturer's instructions. Each freshly mixed cements were condensed into twenty separate wells using separate amalgam carriers and condensers. Excess material was removed with a flat plastic instrument from the unsealed surface (Surface a). All the samples were allowed to set. The sheets containing MTA, Biodentine and Pozzolan cement were then divided into smaller separate sheets. The subgroups were formed & labelled M1, M2, M3, M4, B1, B2, B3, B4, P1, P2, P3 and P4 containing 5 samples each (n= 5).[2]

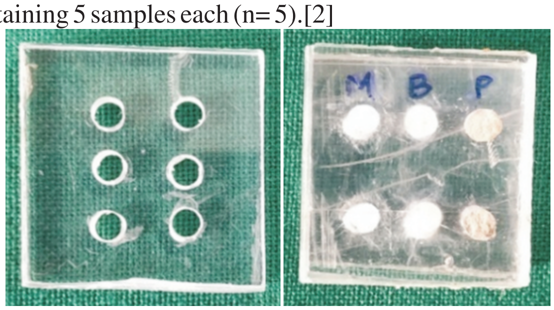

Fig 2: Acrylic sheets with materials condensed into the wells

Following eight subgroups were formed to evaluate the effect of 5% NaOCl and 17% EDTA on different test materials:

MTAA
1. 5% NaOCl on microhardness after 24hrs.(M1)
2. 17% EDTA on microhardness after 24hrs.(M2)
3. 5% NaOCl on microhardness after 8days(M3)
4. 17% EDTA on microhardness after 8 days.(M4)

Biodentine
5. 5% NaOCl on microhardness after 24hrs.(B1)
6. 17% EDTA on microhardness after 24hrs.(B2)
7. 5% NaOCl on microhardness after 8 adys.(B3)
8. 17% EDTA on microhardness after 8 days.(B4)

Pozzolan cement
9. 5% NaOCl on microhardness after 24hrs.(P1)
10. 17% EDTA on microhardness after 24hrs.(P2)
11. 5% NaOCl on microhardness after 8 days.(P3)
12. 17% EDTA on microhardness after 8 days.(P4)

All the samples were kept in an incubator at 37°C and 100% relative humidity for 24 h to set. After 24hrs they were blotted dry with sterile gauze. The surfaces a and b of all the samples were wet-polished with silicon carbide-based sandpapers in the order of 600, 1200 and 2000 grit, with minimal hand pressure, to remove surface scratches. The surface debris was gently removed with sterile guaze. An initial Vickers microhardness test reading (HMV Micro Hardness Tester, Shimadzu)(Fig 3) was then taken as a baseline reading for all the prepared samples on Surface a. The samples from the sub-groups M3, M4, B3, B4, P3 and P4 were kept in the 37°C incubator at 100% relative humidity for another 7 days.[2]

The samples from the sub-groups M1, B1 and P1 were exposed to 5% NaOCl, and those from M2, B2 and P2 were exposed to 17% EDTA. Exposure was performed by immersing the acrylic sheets into the respective solutions for a duration of 10 min. Surface b of M1, M2, B1, B2, P1 and P2 samples were tested for their microhardness.[2]

The testing was repeated after 7 days, on the surface b, with samples from the subgroups M3, B3 and P3 after exposure to 5% NaOCl , and those from M4, B4 and P4 after exposure to 17% EDTA irrigation at the 8-day mark.

The surface microhardness values of all the samples were measured with a digital Vickers hardness tester. Indentations were produced under a uniform setting of a 100-g (0.1 HV) load applied over a 15-s dwell time. On each sample, three indentations of more than 1 mm apart were made on the polished surface with a sharp-pointed, square pyramid-shaped diamond indenter.

Fig 3: Sample placed in the HMV microhardness tester

The diamond-shaped indentations(Fig 4) were examined with an optical microscope equipped with an image analysis software, to enable accurate measurement of their diagonals. The average length of the two diagonals recorded for each indentation was used to calculate the microhardness value. The Vicker's Hardness Number was digitally calibrated. Three measurements were made on the surface of each specimen, and the mean value was taken as the hardness of that particular specimen.[2]

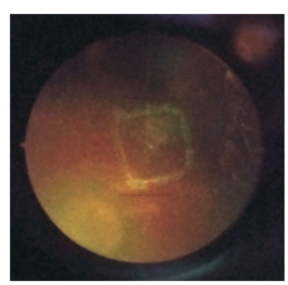

Fig 4: Indentation produced Statistical Analysis

Mean values and standard deviations of each material were recorded. Microhardness differences between the different groups were examined for statistical significance using the one-way analysis of variance (ANOVA) and Tukey's post hoc test was used for comparision between 2 groups.

Results

Table 1 and figure 5 illustrates the analysis of mean Vicker's hardness values for the tested materials at various time points.

	MTA Angelus	Bio dentine	Pozzolan cement
Baseline	81.52±1.94	42.544±5.28	27.44±0.96
NaOCl 24hr	75.66±2.31	25.64±3.43	26.94±0.24
EDTA 24hr	48.38±1.91	37.26±3.01	24.1±2.22
NaOCl day 8	76.96±3.45	72.24±8.75	67.92±9.65
EDTA day 8	52.04±2.52	39.66±1.6	58.92±6.98

Table 1: Analysis of Vicker's microhardness dat a

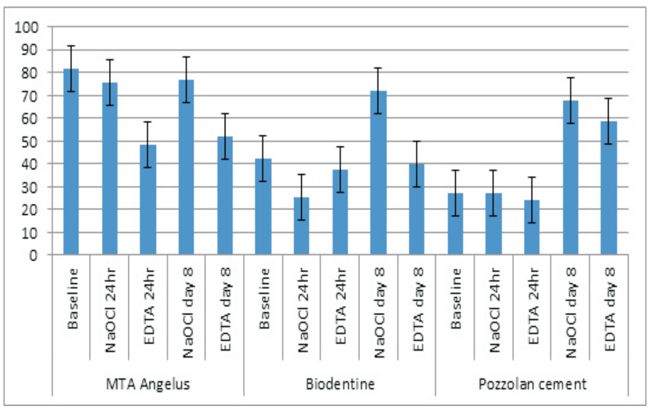

Fig 5: Graphical representation of the mean microhardness values and standard deviations of the tested materials at baseline and after irrigation.

At baseline, MTAA exhibited statistically significant higher microhardness than both biodentine and pozzolan cement. The mean microhardness of MTAA is the highest and that of pozzolan is lowest at baseline.

At 24-hr, NaOCl exposed MTAA and Biodentine showed significantly lowered microhardness values w.r.t. their values at baseline. However, the reduction in the microhardness of NaOCl exposed pozzolan cement at 24hr was not significant. Also, the microhardness of MTAA was significantly higher than biodentine and pozzolan cement. But there was no significant difference between NaOCl exposed Biodentine and Pozzolan cement at 24 hrs.

At 24hr, EDTA exposed MTAA and pozzolan cement showed significant reduction in the microhardness values as compared to baseline values while reduction in values of biodentine was non significant from its baseline values. There was highly significant difference in the microhardness values of EDTA exposed MTAA, Biodentine and Pozzolan cement at 24 hrs. Value of MTAA being the highest followed by Biodentine and then Pozzolan having the lowest value.

At 8day, NaOCl exposed MTAA showed significant decrease in the microhardness values from its baseline values while biodentine and pozzolan showed an increase in the microhardness values. NaOCl exposed MTAA showed highest microhardness scores on the 8day followed by biodentine and then pozzolan.

On 8day the EDTA exposed MTAA and biodentine showed decrease in the microhardness values from their baseline values. Pozzolan showed a significant increase in the mean microhardness values as compared to the baseline values. Among all the three cements, MTAA showed the highest value with significant difference with biodentine but the difference with pozzolan was not significant. Among biodentine and pozzolan cement, pozzolan showed significantly higher microhardness from biodentine.

Discussion:

The prognosis of perforation repair depends on many factors, such as the time delay before perforation repair. The delay of furcal perforation repair can cause microbial contamination of the defect resulting in endo-perio lesion. Furcal perforation should be repaired as soon as possible, before proceeding with the root canal treatment to favor the outcome of the endodontic treatment.[13] An ideal perforation repair material should remain in place under dislodging forces, such as mechanical loads of occlusion or the condensation of restorative materials over it and provide a tight seal between the oral environment and periradicular tissues.[9] The present study evaluated the effect of irrigating solutions namely sodium hypochlorite and EDTA on the microhardness of MTAA, Biodentine and Pozzolan cement(Endocem MTA) after 24hrs and 8 days.

In this study, a reduction in microhardness of MTAA was found on irrigation with NaOCl after 24hrs and 8days. Calcium silicate hydrates (C-S-H) and calcium hydroxide are products of MTA hydration. Reduction of microhardness on MTAA after NaOCl irrigation at 24 h may be attributed to its ability to inhibit calcium hydroxide formation, and hence affecting its hydration. Given that NaOCl affects the microhardness of MTA, it can be inferred that NaOCl inhibits the calcium hydroxide formation by MTA even after 7 days. Several studies have shown that MTA sets within 6 h but it further maturates beyond that, as it was noted that calcium hydroxide production peaks at 7 days, and potentially continues onwards. Therefore, it can be inferred that the setting hydration reaction continues beyond 6 hours.[2]

With EDTA, there was significant reduction in the microhardness values after 24 hrs which further reduced on irrigation after 8days. During hydration, EDTA chelates the calcium ions released by MTA, hence interrupting the precipitation of C-S-H hydration product. This likely contributed to the reduction of microhardness in MTAA, as chelation will cause calcium hydroxide to be lost from the set MTAA. Furthermore, the subsequent dissolution of some of the MTA's C-S-H structure would likely create a surface etching effect.[2]

This study did not show a significant difference between MTAA microhardness at 24 h and 8 days. As the average microhardness for 8 days is higher, it is possible that a statistical difference would have been found if the sample size of this study was larger. Hence, it is recommended to use NaOCl before MTA placement or to wait at least a week before irrigating NaOCl once MTA is placed.

Biodentine showed a reduction in the microhardness on irrigation with NaOCl after 24 hrs. So, it can be inferred that the irrigation with NaOCl interferes with the setting of biodentine that gives a reduced microhardness value after 24 hrs. An increase in the microhardness values was seen after 8 days. The setting time of Biodentine was determined as 45 minutes by Grech et al.[17] while Villat et al. in their study found that at least 14 days were necessary for the calcium silicate based cement to set.[18] So, the increase in microhardness after 8days suggests a better set material after 8days. Therefore, irrigation in a root perforation repaired with biodentine should be delayed atleast for a weak for better results.

On irrigation with 17% EDTA, after 24 hrs, biodentine did not show a significant difference from its baseline values. But the average value is lesser on irrigation after 24 hrs. It is possible that a statistical difference would have been found if the sample size of this study was larger. However, an increase in the microhardness was seen on irrigation after 8days but the values were still less than the baseline values. This may be attributed to chelation of the calcium ions released from BD by EDTA during hydration which lowers its hardness value due to poor crystallization.19

Endocem MTA is similar to MTA but is a fast setting material. Its setting time as reported by Choi et al. is 4 minutes± 30 seconds.[20] The microhardness after 24 hrs on irrigation with NaOCl was found to be nonsignificantly reduced but here also a larger sample size may help us to get the significant results. But after 8 days, on irrigation with NaOCl, it showed significantly higher microhardness values. Jang GY et al.[21] showed that Endocem MTA had higher washout resistance compared with white MTA in the presence of various root canal irrigants including saline, sodium hypochlorite, and chlorhexidine in a furcal perforation model. Shin et al.[22] concluded that Endocem MTA showed higher washout resistance characteristics compared with ProRoot MTA and MTA Angelus with much shorter setting time. In this study also, NaOCl did not significantly reduce the microhardness of endocem MTA after 24 hrs. also, the values were higher after 8days.

With EDTA irrigation after 24 hrs, Endocem MTA showed lesser microhardness scores than the baseline values. But the microohardness values increased after 8days on irrigation. This may be due to its faster setting. Endocem MTA consists of small particles of pozzolan cement to increase the surface contact with the mixing water and provide rapid setting.[23]

Conclusion

Changes in microhardness of MTAA, Biodentine and Pozzolan cement(Endocem MTA) were associated with the time for which the materials are allowed to set as well as the irrigating agent used. Both, NaOCl and EDTA have a detrimental effect on microhardness when the materials are setting. For all the three materials, the final microhardness after exposure to EDTA or NaOCl is improved if exposure to irrigants is made after 8 days of setting instead of 1 day of setting. So, clinicians should allow sufficient time for the perforation materials to set before proceeding with chemomechanical preparation. Also, as these irrigants affect the setting reactions of these materials, EDTA and NaOCl should be flushed from the canals and chamber before these materials are placed for perforation repair into position. Alternatively, chemomechanical preparation should be completed prior to placing MTA, Biodentine or Endocem MTA so that irrigation is not performed after the immediate placement of either material.

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